137 CHARACTERISTICS OF INDUCED INSTABILITY AT A MOUSE TANDEM REPEAT LOCUS. Polyzos AA[1], Parfett CL[1], Healy C[1], Yu T[1], Douglas G[1], Yauk CL[1]. [1]Environmental Health Centre, Health Canada, Tunney's Pasture, 0803A, Ottawa, ON, K1A 0L2, Canada.

Expanded simple tandem repeat (ESTR) loci are non-coding DNA region in the mouse genome that show a high level of instability resulting from loss or gains of repeat units but with several characteristics differing from other classes of tandemly repeated DNA. The frequency and extent of both induced and spontaneous mutations at ESTR loci such as *Ms6-hm* have facilitated their detection using PCR methods. Analysis of pedigrees or direct amplification of single DNA molecules (individual ESTR alleles) has previously indicated that *Ms6-hm* is unstable in both mouse germline and somatic cells. Mutation levels increase when premeiotic and meiotically active germ cells are treated to genotoxic insults (such as ionizing radiation or direct DNA alkylation (using ENU)) resulting in common profiles of size alterations to those of uninduced mutations. This might be indicative of the participation in induced instability of a non-meiotic mitotically associated process such as replication slippage. The link between these effects and genotoxicity is indirect or epigenetic. ESTR instability in cultured murine cells from both genotoxic and non-genotoxic carcinogens (ionizing radiation, DNA alkylation (ENU), oxidative stress (xanthine oxidase generated oxygen radicals) and TPA) has been investigated to probe induced mutation in cells not undergoing meiosis.

138 SEMI-QUANTITATION OF POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DNA ADDUCTS IN HUMAN CERVIX BY IMMUNOHISTOCHEMISTRY AND THE AUTOMATED CELLULAR IMAGING SYSTEM (ACIS). Pratt MM[1], Castle PE[2], Schiffman M[2], Glass AG[3], Scott DR[3], Rush BB[3], Olivero OA[1], Poirier MC[1]. [1]Carcinogen-DNA Interactions Section, Center for Cancer Research, NCI, NIH, Bethesda, MD. [2]Division of Cancer Epidemiology and Genetics, NCI, NIH, DHHS, Bethesda, MD. [3]Northwest Kaiser Permanente, Portland, OR.

Epidemiological studies have shown that, among women with cancer-associated HPV-infection, smoking may increase the cervical cancer risk an additional 2-4-fold (Castle et al., JNCI, 94:1406, 2002). It is therefore of interest to examine human cervical tissue for PAH-DNA adduct formation. Previously, antiserum elicited against DNA modified with anti (+/-)r-7,t-8-dihydroxy-t-9,10-epoxy-benzo-[a]pyrene (BPDE) was validated for semi-quantitative immunohistochemistry (IHC) of human esophagus using the ACIS (van Gijssel et al., CEBP 11:1622, 2002). Here we report optimization of IHC conditions for PAH-DNA adduct visualization in human cervical biopsy tissue. In order to prepare an internal standard, a low-dose BPDE-exposed human keratinocyte standard curve was generated using *in vitro* cultured human cervical keratinocytes exposed for 1 hr to 0, 0.053, 0.153, or 0.310 uM BPDE. Keratinocytes showed parallel dose-dependent increases in BPDE-DNA adducts by IHC/ACIS analysis and quantitative chemiluminescence immunoassay (CIA). In a pilot study, the presence of nuclear PAH-DNA staining was demonstrated in 2 human cervical biopsy samples from HPV-infected women. In these samples the PAH-DNA staining, visible with BPDE-DNA antiserum, was reduced 82 and 89% in parallel sections stained with specific antiserum that had previously been absorbed with the BPDE-DNA immunogen. ACIS values for specific antiserum staining were 12.2 and 6.9 arbitrary units (AUs)/nucleus, compared to 1.4 and 1.2, respectively, for parallel sections stained with absorbed serum. Therefore, the observed nuclear staining is specific for PAH-DNA adduct formation. Further studies will explore the usefulness of PAH-DNA adduct formation as an indicator of cervical cancer risk in smoking women with cancer-associated HPV infection.

139 INHIBITION OF INDUCIBLE GENE EXPRESSION BY CHROMIUM. Puga A[1]. 1Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0056.

Co-contamination with complex mixtures of carcinogenic metals, such as chromium, and polycyclic aromatic hydrocarbons is a common environmental problem with multiple biological consequences. Chromium exposure alters inducible gene expression, forms chromium-DNA adducts and chromium-DNA cross-links, and disrupts transcriptional activator/coactivator complexes. We have previously shown that exposure of mouse hepatoma Hepa-1 cells to chromate inhibits the induction of the *Cyp1a1* and *Nqo1* genes by dioxin. We have tested the hypothesis that chromium blocks gene expression by interfering with the assembly of productive transcriptional complexes at the promoter of inducible genes. To this end, we have studied the effects of chromium on the expression of genes induced by B[a]P, another aryl hydrocarbon receptor (AHR) agonist, and characterized the disruption of Cyp1a1 transcriptional induction by chromium. Gene expression profiling using high density microarray analysis revealed that the inhibitory effect of chromium on B[a]P-dependent gene induction was generalized, affecting the induction of over 50 different genes involved in a variety of signaling transduction pathways. The inhibitory effect of chromium on Cyp1a1 transcription was found to depend on the presence of promoter-proximal sequences and not on the cis-acting enhancer sequences that bind the AHR complex. Using transient reporter assays and Chromatin immunoprecipitation analyses, we found that chromium prevented the B[a]P-dependent release of histone deacetylase-1 from Cyp1a1 chromatin and blocked p300 recruitment. These results provide a mechanistic explanation for the observation that chromium inhibits inducible, but not constitutive gene expression.

140 CONDITIONAL EXPRESSION OF HOGG1 IN MITOCHONDRIA IMPROVES MITOCHONDRIAL REPAIR OF NO-INDUCED DNA DAMAGE AND ENHANCES CELL SURVIVAL. Rachek LI[1], LeDoux SP[1], Wilson GL[1]. 1Department of Cell Biology and Neuroscience, College of Medicine, University of South Alabama, Mobile, Alabama 36688.

Nitric oxide (NO), a free radical inorganic gas, has been shown to have a biphasic action on cell killing with low concentrations exhibiting a protective effect against cell death, whereas higher concentrations are toxic. There is good evidence that NO is genotoxic, i.e. it can cause DNA damage and mutations. Recently, it has been reported that NO can be generated by mitochondria. Mutations in mitochondrial DNA (mtDNA) have been associated with a variety of diseases including diabetes, ischemic heart disease, Parkinson's disease, Alzheimer's disease, and the normal process of aging. These mutations could be partially the result of either increased damage to mtDNA or decreased repair of this damage, or a combination of both factors. It has been shown that NO damages mtDNA to a greater extent than nuclear DNA. Previously, we have reported that conditional targeting the DNA repair protein hOGG1 to mitochondria augmented mtDNA repair of oxidative damage in this organelle and enhanced cellular survival. In the current studies we used HeLa TetOff/MTS-OOG1 transfected cells that conditionally expressed OGG1 in mitochondria in the absence of the doxycycline (Dox-) to evaluate the effect of additional hOGG1 expression on the repair of NO-induced mtDNA damage and cell survival. Dox ± HeLa TetOff/MTS-OOG1 and vector transfectants were exposed to NO produced by the rapid decomposition of 1-propanamine, 3-(2-hydroxy-2-nitroso-1-propylhydra-zino) (PAPA NONOate) and the resultant damage to mtDNA was determined by quantitative Southern blot analysis. Functional studies revealed that cells expressing recombinant OGG1 were more proficient at repairing NO-induced damage in their mtDNA, and this increased repair led to increased cellular survival following NO-exposure.